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Ancient DNA Analysis of Fremont Amerindians of the Great Salt Lake Wetlands

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ABSTRACT Skeletal remains of 47 individuals from the Great Salt Lake Wetlands, affiliated principally with Bear River (A.D. 400–1000) and Levee Phase (A.D. 1000–1350) Fremont cultural elements, were assessed for four mitochondrial DNA (mtDNA) markers that, in particular association, define four haplogroups (A, B, C, and D) widely shared among contemporary Amerindian groups. The most striking result is the absence of haplogroup A in this Fremont series, despite its predominance in contemporary Amerindian groups. Additionally, haplogroup B, defined by the presence of a 9bp deletion in region V, is present at the moderately high frequency of 60%. Haplogroups C and D are present at low frequencies. An additional haplotype, "N," observed in some modern populations and two other prehistoric samples, is also present in this Fremont skeletal collection.

GREAT SALT LAKE WETLANDS

The Great Salt Lake (GSL) wetlands are known to have been an area of intense human activity during the prehistory of the Great Basin (Fig. 1). Prehistoric sites attributed to the Fremont cultural horizon are well represented, with over 500 identified sites between Brigham City and Ogden, Utah, alone (Simms et al., 1991; Fawcett and Simms, 1993). Traditionally, the Fremont have been defined by contrast with the roughly contemporaneous Anasazi cultures farther south. Fremont culture is usually distinguished from the Anasazi by a unique set of archaeological traits such as moccasin and pottery styles, clay figurines, a geographically constrained type of maize, and the lack of a formal kiva (Wormington, 1955; Jennings, 1978; Madsen, 1979; Cordell, 1984; Simms, 1986). On the basis of pottery core areas and an implied shared artifact tradition, Fremont groups have been classified into five regional variants: the GSL, Uinta, San Rafael, Sevier, and Parawan (Marwitt, 1970; Madsen, 1979, 1989; Cordell, 1984). While the Fremont sites in northern Utah are clearly distinguishable from the Anasazi sites of the Colorado Plateau and San Juan Basin, Fremont groups geographically closer to the Anasazi core area exhibit greater similarity to the Anasazi, in some cases being nearly indistinguishable (Madsen, 1989; Jennings, 1978; Cordell, 1984). This gradient of Fremont material culture traits, and the presumed reliance on maize agriculture, led some archaeologists to suggest that Fremont groups simply represent "splinter" Anasazi bands (Morss, 1931; Gunnerson, 1969; Berry, 1975). Others sought connections with southward migrating Athapaskan groups (Aikens, 1966; Sharrock, 1966), while still others saw Fremont origins in the earlier Archaic peoples (Wormington, 1955; Jennings et al., 1956; Aikens, 1970; Marwitt, 1970). More recently, distinc-

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tions between the regional Fremont variants have become less clear as archaeological investigations continue to reveal unexpected diversity in subsistence, settlement pattern, and artifact assemblages within each of the variants, resulting in substantial overlap of material culture markers between them (Simms, 1986, 1994a,b).

The ultimate fate of the Fremont culture is as enigmatic as its origin. Fremont pottery and artifacts begin to disappear from the archaeological record around A.D. 1300 and are replaced by an archaeological tradition recognized as Paiute-Shoshoni (Madsen, 1975; Young and Bettinger, 1992). This abrupt cultural transition, coupled with linguistic data (Lamb, 1958; Miller et al., 1971), raised numerous questions regarding Fremont identity, resulting in a model of Fremont replacement by Numic-speaking migrants to the Great Basin during the 14th century.

The skeletal sample analyzed in this study is affiliated with the GSL variant of the Fremont (Simms et al., 1991; Fawcett and Simms, 1993), dating primarily from the Bear River and Levee phases of GSL Fremont cultural context, and spanning a maximum age range of A.D. 252–1296. Thus, the sample under study predates the hypothesized Numic expansion into the area. Originally viewed as settled agriculturists, the GSL Fremont peoples are now known to have practiced a dimorphic lifeway. Archaeological evidence indicates that the classic, relatively large, settled farming communities coexisted with small, highly mobile groups of gatherers and hunters (Simms, 1986, 1994b; Simms et al., 1991; Coltrain, 1993, 1994). Areas east of the GSL wetlands are believed to have served as large agricultural tracts. Unfortunately, the extent of these arable lands in prehistory cannot be known due to site destruction by contemporary urbanization, although a number of such sites have been well documented (Simms and Stuart, 1993).

AMERINDIAN mtDNA HAPLOGROUPS

Recent molecular genetic investigations of native populations of the New World reveal the presence of at least four mtDNA haplogroups (Schurr et al., 1990; Ward et al., 1991; Torroni et al., 1992, 1993a,b). In modern Amerindian populations, these haplogroups are defined by combinations of presence and absence of a minimum of four specific mtDNA markers (Table 1; Schurr et al., 1990).

The GSL skeletal set was characterized for the frequencies of these four markers by the use of enzymatic amplification with appropriate primer pairs (Table 2). Minimally, haplogroup A is defined by a *Hae* III site at np 663; haplogroup B by an intergenic 9 bp deletion between the cytochrome oxidase II and lysine tRNA genes (region V; Cann and Wilson, 1983; Wrischnik et al., 1987); haplogroup C by a *Hinc* II site loss at np 13259; and haplogroup D by loss of an *Alu* I site at np 5176 (Table 1). Stone and Stoneking (1993) recently reported a single ancient sample that did not conform to any of these four defined groups. This specimen, lacking the deletion and the *Hae* III site, but possessing both the *Alu* I and *Hinc* II sites, was termed haplotype N by these authors.

MATERIALS AND METHODS

Populations sampled

Bone samples from 47 individually dated burials were used in this study, representing GSL Fremont samples from Bear River and Levee phases excavated in 1990–1993. Many bone samples destined for DNA analyses were excavated by gloved archaeologists, immediately isolated in plastic bags, and left unwashed. Field recovery procedures for the skeletal materials included here are detailed in Simms et al. (1991). The remaining samples were selected for molecular analysis by two of us (RLP and DHO'R) subsequent to excavation and curation. Clearly, these samples do not constitute a population as generally defined in population biology. However, we believe it is appropriate to view them as representative of a continuous population due to the relatively short time frame from which they come, the essentially identical geographic spot from which all samples were recovered, and the uniform material culture characterizing all sites yielding human remains. Moreover, the major, hypothesized population movement/replacement in this geographic area is thought to postdate these

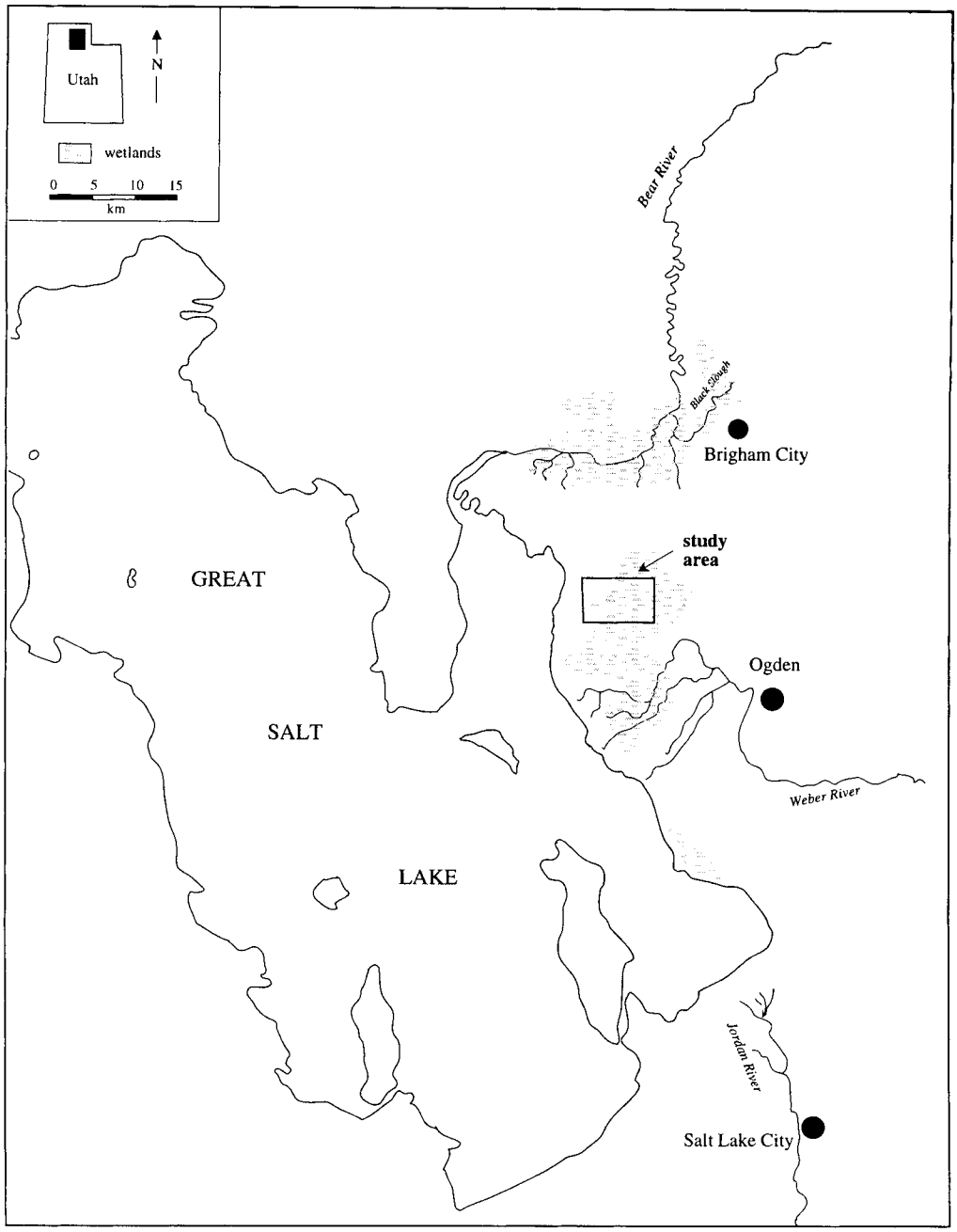

Fig. 1. Geographic location of the Great Salt Lake wetlands and origin of samples used in this study.

samples. While only larger numbers of samples and more extensive molecular screening can adequately test the hypothesis, it is here assumed that the individual samples represent individuals from a continuous, local population resident on the eastern margin of the Great Salt Lake during this period of prehistory. Such an interpretation is quite

TABLE 1. Distribution of markers in Amerindian mtDNA lineages

mtDNA lineage	Region V 9 bp deletion	*Hae*III np663	*Alu*I np5176	*Hinc*II np13259
A	–	+	+	+
B	+	–	+	+
C	–	–	+	–
D	–	–	–	+
N	–	–	+	+

Presence of a marker indicated by a +, and its absence by –

consistent with a wealth of archaeological evidence.

Extracts

DNA was extracted following a modification of the procedures of Hagelberg et al. (1991). Bone was decalcified in 10 ml 0.5 M EDTA pH 8.5 for 72 hours with a fresh change of EDTA at 24-hour intervals. Since it is unlikely that nucleases remain active in ancient tissues, and since EDTA inhibits proteinase K (PK) activity, EDTA was removed before digestion with PK by three successive washes with sterile ddH_2O. Samples were digested overnight in 2 ml of PK digest buffer (50 mM Tris pH 8.0, 1 mM $CaCl_2$, 1 mM DTT, 0.5% tween 20, and 1 mg/ml PK) at 55°C, phenol extracted twice, chloroform/isoamyl alcohol (24:1) extracted once, concentrated on amicon 30 columns and rinsed with sterile ddH_2O. Resulting volumes were brought to 200 µl with ddH_2O and etoh precipitated in the presence of 2.3 M ammonium acetate and 10 mM $MgCl_2$, rinsed with 80% etoh, desiccated and redissolved in 1/50thx DNA buffer (3 mM NaCl, 3 mM Tris pH 8.0, and 0.30 mM EDTA). Eliminating EDTA from the PK solution and adding $CaCl_2$ to a final concentration of 1 mM increases PK activity (Sambrook et al., 1989), increasing nucleic acid yields and making it unnecessary to powder bone samples, as small, intact pieces digest to completion overnight. Confirmation of results from initial amplifications were generally achieved with replicate extracts obtained as described above. Additionally, second extract samples were soaked in 5% sodium hypochlorite for 20 minutes after being cross-linked with UV light (254 nm) to further ensure elimination of contemporary nucleic acid contaminants.

All extractions were completed using sterile disposable lab ware and autoclaved and dedicated reagents by masked and coated laboratory personnel wearing UV cross-linked gloves. All procedures were performed in dedicated areas sterilized with bleach and/or cross-linked with UV light to eliminate contamination with modern nucleic acids. Additionally, at least one negative extract control was included in each extraction procedure in order to facilitate detection of the introduction of modern contaminants.

PCR amplifications

Dedicated pipettors, sterile barrier tips, sterile reagents, and cross-linked gloves were used in PCR set-up. Reactions were prepared in a Cleansphere (Safetech) controlled environment, purged with UV light (254 nm) and continuously flushed with filtered air, in a separate room, removed from the thermal cycler. Both positive and negative controls were also employed with each amplification. Negative controls included the blank extract control as well as reaction tubes to which no DNA samples were added in order to provide a constant check on the purity of the PCR reagents. Positive controls alternated between HeLa cell extracts and DNA samples derived from laboratory personnel.

Amplifications were typically done in 25 µl volumes using 2 units Deep Vent (exonuclease$^-$) polymerase (DVP$^-$; New England Biolabs), 1× buffer supplied by the manufacturer as 10× stock, 100 µg/ml BSA (non-acetylated), 200 nM dNTPs, 2 mM $MgSO_4$, 0.25 µm each primer, and 5 µl ancient DNA extract diluted 1:10. To further ensure elimination of spurious DNA contamination, reaction mixes were UV cross-linked at 254 nm for 10 minutes (Sarker and Sommer, 1990) prior to the addition of DVP$^-$ and ancient template. All manipulations were done on ice with tubes placed in a 95°C thermal cycler to achieve a "hot start" effect (Chou et al., 1992). Primers identifying the four Amerindian mtDNA haplogroups (Stone and Stoneking, 1993; Table 2), with the exception of region V primers, were annealed at 55°C, for 1 minute, extended at 72°C for 15 seconds, and denatured at 95°C for 35 seconds through 40 cycles. Amplifications of region

TABLE 2. Primers employed in Fremont aDNA analysis

Marker	Primers	Primer sequence (5'–3')
Region V Del.	L8215	ACAGTTTTCATGCCCATCGTC
	H8297	ATGCTAAGTTAGCTTTACAG
Hae III: 663	L611	ACCTCCTCAAAGCAATACACTG
	H743	GTGCTTGATGCTTGTTCCTTTTG
Alu I: 5176	L5120	TAACTACTACCGCATTCCTA
	H5230	AAAGCCGGTTAGCGGGGGCA
Hinc II: 13259	L13232	CGCCCTTACACAAAATGACATCAA
	H13393	TCCTATTTTTCGAATATCTTGTTC

Primers are as given in Stone and Stoneking (1993). L and H refer to the light and heavy chains of the mtDNA molecule, respectively. Numbers indicate nucleotide position according to Anderson et al. (1981).

V were annealed at 59°C. All amplifications were treated with an initial denaturing step at 95°C for 5 minutes prior to the first round of amplification.

Aliquots (5 μl) of the PCR reaction product were resolved on 3–4%, 3:1 NuSieve gels to determine results. Products harboring restriction site polymorphisms were re-extracted and etoh precipitated, washed and redissolved in ddH_2O. Samples were restricted in 20 μl volumes according to manufacturer's recommendations (BRL), with 10-fold enzyme excess. Digests were resolved on 3–4%, 3:1 NuSieve gels. The region V length polymorphism was scored directly on the ethidium bromide stained PCR product gels.

RESULTS

Initial attempts to amplify GSL wetlands bone extracts using published protocols were disappointing. PCR reactions using increasing amounts of *Taq* polymerase (Pääbo et al., 1988) and varying concentrations of BSA (Hagelberg et al., 1989), while trying to dilute contaminants found in ancient DNA extracts (Pääbo, 1990), produced target replication in positive controls (HeLa cell DNA), but only occasionally in ancient samples. The wetlands from which these burials were excavated have been periodically flushed with Great Salt Lake lake water as a result of fluctuations in lake levels over the past several thousand years. We speculate that the high salt content (18–27%) of the lake water and/or the mechanical effects of repeated flushing of the burial sites with lake water could have released DNA from the hydroxyapatite matrix of the bone, reducing extractable amounts of DNA. Nevertheless, collagen yields, which have been proposed as an indicator of DNA preservation in bone (Tuross, 1994), appeared acceptable from the GSL extracts (% collagen yield range = 3.3–25.5; mean = 16.7; Coltrain, 1994). To access seemingly present nucleic acids, EDTA was eliminated from the PK buffer, with $CaCl_2$ added to increase PK activity. In addition, extracts were precipitated in the presence of 2.3 M ammonium acetate, which is known to eliminate enzyme inhibiting contaminants. Also, DVP^- replaced *Taq* I as the amplification enzyme. Manufacturer specifications indicate that DVP^- has a higher K_m value for DNA substrate and greater sensitivity for dNTPs than *Taq* I. Additionally, DVP^- has a longer half-life at 95°C and tolerates high concentrations of BSA, which has been shown to reduce polymerase inhibition in PCR amplifications with ancient substrate (Pääbo et al., 1988; Hagelberg and Clegg, 1991).

Using these extraction and amplification modifications, 43 of 47 samples from the GSL wetlands skeletal series amplified with at least one of the four primer sets that detect the four Amerindian genetic markers. Four extracts failed to amplify with any of the primer sets. Typically, PCR results were either replicated in separate amplification reactions from the same extract, or duplicated in separate extracts, often both. Overall success rates for each primer set are: 85% (40/47) for region V; 75% (35/47) for *Alu* I; 79% (37/47) for *Hae* III; and 57% (27/47) for *Hinc* II.

Of the 40 samples that successfully amplified with region V primers, 60% (24/40) possessed the 9bp deletion (haplogroup B, Table 3). The *Hae* III restriction site at np 663 is

TABLE 3. Great Salt Lake wetlands skeletal analysis: Marker frequencies and haplogroup distribution

Marker	*Hae* III 663 (+)	9 bp deletion	*Hinc* II 13259 (−)	*Alu* I 5176 (−)
Haplogroup	(A)	(B)	(C)	(D)
Counts	0/37	24/40	4/27	2/35
Marker frequency	0%	60%	15%	6%
Haplogroup frequency	0%	73%	13%	7%

See text for explanation of disparity between marker and haplogroup frequencies.

absent in these samples (Table 3). None of the 37 samples which amplified with the *Hae* III primer pair restricted with the *Hae* III enzyme. In contrast, up to 5 μg of control template (pBR322) easily restricted to completion under the same conditions. The absence of the *Hinc* II site at np 13259, associated with haplogroup C, was observed in four of 27 successful amplifications (15%), while the lack of the *Alu* I site at np 5176, the marker for haplogroup D, occurred in two individuals (6%). Haplotype "N" (Table 1) was positively identified in two GSL individuals. Seven specimens could not be unambiguously placed in a haplogroup.

As a result of the inability to resolve individual markers for some specimens, specification of haplogroup frequencies in these data is somewhat problematic. If we identify haplogroups by the presence or absence of an individual defining marker, then haplogroup frequencies are coincident with the marker frequencies given in Table 3. However, haplogroups are properly defined by the co-occurrence of multiple markers, and altering the state of any one of them may change assessment of lineage membership. In the skeletal series analyzed here, 21 samples amplified with all four primer sets. An additional nine, although lacking one or more PCR products, could be assigned haplogroup membership assuming that no previously unidentified haplogroups existed in these samples. Thus, relatively unambiguous determination of lineage membership was possible for 30 individuals. Of these 30 informative samples, 73% belong to haplogroup B. This is likely an overestimate of the population frequency of this haplogroup since only 60% of the samples possessed the marker that typically defines it. Haplogroup C comprises 13% (4/30) of this reduced sample, and haplogroup D 7% (2/30). Two individuals were unambiguously determined to be haplotype N (7%, not shown in Table 3).

It is worth noting that in addition to the measures and checks listed above to prevent and monitor the introduction of modern DNA contaminants, additional safeguards were also employed. Two fragments from the hypervariable D-loop were amplified in several of these samples, one of 220 bp, and another of 445 bp. While results of sequence analysis of these fragments will be presented elsewhere, it is significant that the amplification yields of these fragments is strongly influenced by fragment size. The smaller of the two fragments amplified in every sample attempted, but the yield was notably reduced from that seen for the smaller fragments resulting from PCR with the primers in Table 2. Additionally, the longer D-loop product was observed in barely half the samples attempted, and in those amplified only weakly. Moreover, a 387 bp fragment from exon IV of the human lectin gene in the nuclear genome amplified in only two samples, and the yields in these two samples were notably reduced from that in modern controls (data not shown). This observed correlation between fragment size or copy number and amplification yield may be taken as evidence that the products under study are, in fact, from ancient template rather than modern contaminants (e.g., Handt et al., 1994). Such observations, coupled with the stringent contamination control procedures outlined above, engender confidence that the results reported here are not spurious as a result of contamination.

DISCUSSION

The absence of haplogroup A in this Great Basin skeletal series is somewhat surprising since it is the most common haplogroup ob-

served in modern Amerindian populations (e.g., Torroni et al., 1993a; Bailliet et al., 1994). This haplogroup is absent in some South American populations (Yanomamo and Wapishana), and is also known to be absent in a number of native Siberian populations (Torroni et al., 1993b). Among native North American populations, the Pima of the Southwest have a notably low frequency of haplogroup A (6.7%, Torroni et al., 1993a), while Northern Athapaskan populations, in contrast, are essentially fixed for this haplogroup.

The relatively high frequency of the 9 bp deletion observed in this pre-Columbian skeletal series is also of interest. The frequency of this marker ranges from absent in circumarctic peoples and Northern Athapaskans (Shields et al., 1992, 1993; Torroni et al., 1993a,b), to frequencies exceeding 60% in others (e.g., 63% in the Yakima, Shields et al., 1993; 71% in the Aymara, Merriwether et al., 1994). Of particular interest is the apparent low frequency of this marker in contemporary Paiute and Shoshone peoples of the Great Basin (14.3%, Lorenz and Smith, 1994). The deletion is polymorphic in most Asian populations, reaching fixation in some coastal populations and Polynesian Islanders. The marker also has been reported in 2,000-year-old samples from Easter Island (Hagelberg et al., 1994). However, high frequencies for the marker in ancient Amerindian samples have not been reported before. It was present at only modest frequencies in the ancient Central and South American populations reported by Merriwether et al., (1994), absent in a single 7,000-year-old sample from Florida (Pääbo, 1988), and present in only 10% of a sample of 50 individuals from an Oneota cemetery in Illinois (ca. A.D. 1300, Stone and Stoneking, 1993).

The observed frequencies of the C and D haplogroups are consistent with frequencies observed in many Amerindian and Siberian populations. It is the rather unusual frequencies of the A and B haplogroups in this Fremont sample that are of principal interest. The absence of the generally common A haplogroup, coupled with the relatively high frequency of the B haplogroup, suggests the action of genetic drift (e.g., Avise et al., 1984; Cavalli-Sforza et al., 1994). This inference is consistent with the view of northern Fremont lifeways derived from archaeological and stable carbon isotope analyses (Simms and Stuart, 1993; Simms, 1994b). Great Salt Lake Fremont populations engaged in some settled agriculture when environmental conditions permitted, and relied on hunting and gathering wild resources at other times. In fact, it now appears likely that these groups pursued both economic strategies simultaneously (Simms, 1994b; Coltrain, 1994). They appear to have been a highly resilient, opportunistic, and mobile population during much of prehistory, although the mobility may well have been locally restricted. This view of comparatively small, mobile groups is quite consistent with an expectation of random loss, or augmentation, of mitochondrial lineage frequencies (e.g., Avise et al., 1984).

Among contemporary Amerinds, frequency of loss of individual haplogroups appears to be highest in South American populations (e.g., Bailliet et al., 1994); consistent with the hypothesis that patterns of genetic variation among these generally small, relatively isolated populations is primarily a reflection of stochastic forces (O'Rourke and Suarez, 1985; Black, 1991; O'Rourke et al., 1992; Cavalli-Sforza et al., 1994). If, in fact, most prehistoric populations were small and subject to random lineage extinction, it may be comparatively easy to document genetic differences between them, but rather more difficult to assess the historical nature of their biological affinities, depending on the antiquity of the archaeological samples and the timing of lineage extinctions. Demonstrating "ancestral exclusion," on the other hand, may be much easier. Such challenges highlight the need for close collaboration between archaeologists and anthropological geneticists in addressing population problems in prehistory.

As noted in the introduction, some archaeologists hypothesized that Fremont origins could be found among the Anasazi, or alternatively, as remnants of the Athapaskan southern migration. While no longer supported by archaeological data, these early suggestions may be refuted with the present molecular data. Modern northern Atha-

paskan populations lack the region V deletion and, hence, haplogroup B. Rather, they are nearly fixed for haplogroup A. The lack of haplogroup A, and high frequency of B, in the Fremont sample effectively precludes Athapaskan ancestry. Additionally, preliminary analysis of molecular variation in a sample of Anasazi from southern Utah (O'Rourke et al., 1996) is suggestive of some genetic distinctiveness between the Anasazi and Fremont. If confirmed, this preliminary finding will be especially interesting since the two samples are geographically proximate and overlap in temporal distribution.

The distinctiveness of Fremont from Anasazi, however, should be viewed with some caution. If, as just suggested, the absence of lineage A among the Fremont, and perhaps lineage D among the Anasazi, as well as the substantial difference in the frequency of lineage B between these groups, reflects the normal processes of lineage extinction and genetic drift, then the observed genetic differences between the samples need not be taken as strong evidence for separate origins. Indeed, both samples have some affinity to modern populations to the south and west. Like the Anasazi examined to date (O'Rourke et al., 1996), modern populations of southern Mexico also lack haplogroup D (Torroni et al., 1994). Unlike most north Amerinds, the Fremont share with the modern Pima (and ancient Anasazi) a very low frequency (or absence) of haplogroup A. Thus, an alternative hypothesis of Fremont origins to those previously offered may be formulated based on these molecular data: the Fremont and Anasazi are genetically related by origin from ancestral populations originally residing farther to the southwest, represented today by modern populations of Mexico and Mesoamerica, and their relatively recent migrants to the U.S. Southwest. Complete testing of these competing hypotheses awaits larger-scale screening of ancient populations, as well as more extensive molecular characterization of existing samples—studies that are now underway.

It is unfortunate that the northern Utah Fremont sample does not encompass the late prehistoric period, typified archaeologically by Paiute-Shoshone affiliated material, since the apparent resolution of the molecular data would be sufficient to finally test the Numic expansion hypothesis for the origin of modern Great Basin Amerindian groups (see Simms, 1994a, for a recent archaeological review and perspective). While it is tempting to view the disparity in the deletion frequency between the Fremont and modern Paiute and Shoshoni peoples (Lorenz and Smith, 1994) as evidence for such an expansion, a full and adequate test of the Numic expansion hypothesis must await a more complete molecular characterization of late prehistoric samples and/or their modern descendants. In this connection it is useful to note that nearly half the samples examined were in spatial association with presumed late prehistoric period ceramic shards. However, each sample has been directly dated by AMS ^{14}C and only one dates to the time period of Fremont-Late Prehistoric transition. This sample possessed the deletion and is, therefore, haplogroup B. The lack of correspondence between cultural affiliation based on spatially associated artifacts and the direct dates, a phenomenon well known to archaeologists, underscores the importance of directly dating individual samples, and emphasizes the complexity of the economies of prehistoric populations and their reflection in the archaeological record (Simms, 1994b; Coltrain, 1994).

Finally, Stone and Stoneking (1993) observed a single haplotype in their study of Oneota skeletons that did not correspond to one of the putative founding haplogroups (A–D). They termed this haplogroup "N." At least two of the GSL Fremont samples are characterized by this haplotype, which is defined by an absence of the region V deletion and the *Hae* III site at np 663, but the presence of the *Alu* I site at np 5176 and *Hinc* II site at 13259. Moreover, one additional sample is likely to be haplotype N, while two others may well be. The inability to unambiguously determine the haplotype of these three specimens derives from the failure to amplify one or more restriction-site-bearing fragments. Stone and Stoneking (1993) note the possibility that the presence of this haplotype in their Oneoto sample may be the result of contamination with non-Amerindian DNA. The same is possible here. However, we believe it unlikely since the haplo-

type has now been observed in multiple ancient Amerind samples and is clearly present in modern Amerindian and Asian populations. Moreover, we have no evidence of contamination in the experiments yielding the data reported here, and considerable evidence, described above, that the data reflect molecular variation in ancient DNA rather than artifacts of contamination. We are, however, continuing to evaluate these results by D-loop sequence analysis to confirm these initial findings.

The constellation of markers here called haplotype N has been observed in several contemporary Amerinds (Torroni et al., 1993a), and Bailliet et al. (1994) recently argued that it should be considered a fifth founding lineage, haplogroup E. Torroni et al. (1993a) attributed these "other" haplotypes to non-Amerind admixture. The presence of this haplotype in multiple samples of pre-Columbian age precludes such admixture as the sole explanation for its presence in modern Amerinds.

Nevertheless, it is not obvious that the N haplotype is actually an additional founding haplogroup. Unlike the other four haplogroups, the N haplotype is not defined by the presence or absence of a specific marker, but rather by the exclusion of the four defined haplogroups *when using only the four markers employed here*. Moreover, the predictable co-occurrence of several markers typical of haplogroups A–D is not seen in modern samples that, using only these four polymorphisms, would be called haplogroup N. It therefore does not seem possible, based on only these markers, to convincingly argue for an additional haplogroup. That must be done with a larger suite of characters in order to rule out the possibility of the N haplotype simply resulting from a new mutation for a single marker within an established haplogroup (Torroni and Wallace, 1995; but cf. Bianchi and Rothhammer, 1995). The presence of this haplotype in at least three pre-Columbian populations (it is also observed in the early Anasazi, O'Rourke et al., 1996) suggests that there was considerable molecular variation in ancient Amerinds, that it could derive from ancestral populations in Asia (cf. Ballinger et al., 1992), and that the restricted set of haplotypes observed in modern populations is probably the result of random extinction of mtDNA lineages in earlier Amerindian populations.

Whether the apparent restriction in contemporary Amerindian mitochondrial lineages is the result of a founder effect at colonization (Wallace et al., 1985; Torroni et al., 1993a) or the product of evolutionary events subsequent to colonization of the Americas (Ward et al., 1991; Szathmary, 1993; Santos et al., 1994) remains to be conclusively demonstrated. However, the present analysis suggests that at least some of the "lost" genetic variability may be the result of relatively recent drift rather than a significant bottleneck at colonization (cf. Cavalli-Sforza et al., 1994). Continued assessment of molecular variation in prehistoric populations appears to be a profitable avenue of research that will ultimately lead to a resolution of this debate. It seems clear that echoes of ancient population movements are archived in the mtDNA of present and past Native Americans. Additionally, it is clear that ancient DNA studies are capable of documenting ancestral-descendant relationships between prehistoric and modern groups, and evaluating biological affinities among pre-Columbian populations. Documentation of ancient patterns of molecular variation will ultimately prove invaluable in clarifying prehistoric population movements and testing archaeologically derived hypotheses. In the present instance, skeletal samples from later time periods, coupled with molecular characterization of contemporary Paiute-Shoshoni populations, will permit testing the Numic expansion hypothesis, while molecular study of skeletal populations from the geographic area between northern Utah and the four-corners area will be critical in understanding the dynamics of regional Fremont populations and their relationship to the Anasazi and other populations of the larger geographic region.

CONCLUSION

The molecular characterization of a Great Salt Lake Fremont skeletal series reveals a population lacking haplogroup A and exhibiting a relatively high frequency of haplogroup B. While the observed haplotype dis-

tributions are most likely the result of drift in small, prehistoric populations, the general similarity to some contemporary U.S. Southwest populations (e.g., Pima) suggests that Fremont origins are plausibly further to the south and west than the archaeological distribution of the Fremont would indicate. Moreover, preliminary comparisons suggest that the Fremont are genetically divergent from the roughly contemporaneous Anasazi (O'Rourke et al., 1996). The presence of an additional haplotype, N, in these samples and the Anasazi suggest that earlier populations may have been characterized by mitochondrial variation comparable to that typically observed in modern Amerindian populations.

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